OCCURRENCE AND DISTRIBUTION OF FECAL INDICATOR BACTERIA WITH RESPECT TO URBAN AND RURAL LAND USES

By

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1. INTRODUCTION

Statement of Problem

In 1972, there was an increasing concern for protecting the public from polluted water which led to the passing of the Federal Water Pollution Control Act. This act later became the Clean Water Act in 1977. The intent was to regulate discharge of pollutants into and around bodies of water in the United States. It gave the Environmental Protection Agency (EPA) the authority to employ certain pollution control programs. This made it unlawful to discharge any contamination into surface waters from any point source pollutant and helped to fund the construction of sewage treatment plants (USEPA, 2002a).

"According to section 303(d) (1)(A) of the Clean Water Act, each state shall identify those waters within its boundaries for which the effluent limitations are not stringent enough to implement any water quality standard (WQS) applicable to such waters." In addition, the Clean Water Act requires all states to establish priority ranking of water quality and to established total maximum daily loads for these waters (Benham, 2006)."

Furthermore, all around the nation there was a growing concern for the protection of recreational waters that people use for a variety of different activities such as swimming, kayaking, rafting, hiking, camping, and fishing. In 1986, the Environmental Protection Agency published ambient water criteria for bacteria (USEPA, 2002a). This

document listed criteria for the safety of the people who are active in and around recreational waters. This established indicators which included *E. coli, Enterococci* and fecal coliform as indicators of the likely presence of human pathogens within these recreational waters. The use of these indicators provides managers with a way to determine the likelihood that human pathogens may be present in recreational waters.

The main avenue of exposure to disease causing organisms in recreational water is through ingestion through mouth, nose, ears, or skin when in direct contact with contaminated water (i.e. swimming). There are certain gastrointestinal disorders that humans may contract when coming in full body contact with these microorganisms. Humans may get infections in their throat, skin or other area that may come in contact with contaminated water. Many of these infections are transmitted from other people participating in recreational activities at the same location.

Individuals who become sick as a result of contact with contaminated water, often do not think their illness is a consequence of swimming in unclean water. Symptoms generally appear a few days after contact with the contaminated water, and many are not severe enough to contact a physician. Symptoms of illness include vomiting, diarrhea, stomach ache, nausea, headache, and fever (USEPA, 2002a).

The possibility of people getting sick may depend on several factors such as the type of pathogen and exposure time. The amount of time that a person is in the water may determine the severity of the illness. The concentration of the pathogens in the body of water will have a serious impact on how sick an individual may become from full body contact.

Regulation

There are numerous factors that can have an effect on the amount of pathogens in a body of water. For example, water quality of a stream can be affected by land use and flow regime. Water monitoring programs are vital for finding potential sources of contamination under different land uses. It is important for agencies to find the proper indicator because monitoring fecal indicators can be costly and time consuming.

Different states use different bacterial indicator species as surrogates for pathogens. Many states also have different criteria and standards for pathogen violation based on indicator species. States set water quality standards depending on the intended uses and protection needed. Indicator species have certain characteristics that may allow them to survive in different environments.

The Oklahoma water quality standard (OWRB, 2007) as shown in Table 1-1 is 200 Colony Forming Units (CFU)/100 ml for fecal coliform, 126 CFU/100 ml for *E. coli* and 33 CFU/100 ml *Enterococci*. No single sample for *E. coli* should exceed 406 CFU/100 ml at a confidence level of 90%. For *Enterococci*, no samples should exceed 106 CFU/100 ml with a confidence level (C.L.) of 90%. Any single sample for fecal coliform should not exceed 400 CFU/100ml during a thirty day period.

For scenic rivers and lakes, the regulations are more stringent. No single sample should exceed 61CFU/100ml in lakes and high use waterbodies for *Enterococci* at a 75% confidence level. For *E. coli*, no single sample shall exceed 23CFU/100ml at a 75% confidence level (ODEQ, 2006). Waters that do not exceed these standards are considered suitable for fishing, swimming and for a healthy ecosystem.

Table 1-1: Oklahoma Recommended Bacterial Standards

Indicator Organisms	Geometric Means (CFU/100ml)	Single Sample 75% C. L. (CFU/100ml)	Single Sample 90% C. L. (CFU/ 100 ml)
Fecal Coliform	200	400	400
E. Coli	126	235	406
Enterococci	33	61	106

Economic Impact

Placing a value on sickness is something that has been debated for quite some time. Excessive amounts of pathogen in streams can cause severe health risks as well as economic losses including large medical cost and loss of productivity. The number of illnesses each year from contaminated water could result in millions of dollars in cost (CDC, 2005). For instance, a Milwaukee outbreak of Cryptosporidiosis resulted in nearly $100 million in medical and productivity cost (Corso, 2003).

Microbial contamination of recreational waters can be very expensive, especially in coastal regions where there is lots of tourism. Many beaches are forced to close due to fecal contamination when waters don't meet EPA standards. The economic cost of beach closing each year may be in the billions (McLellan, 2003). Monitoring these pathogens may also become expensive because it takes lots of labor and time to monitor fecal indicators. Tracking contamination back to the source can also be costly.

Recreational Waters

Recreational waters around the United States are affected by elevated levels of fecal contamination. Recreational waters comprise ponds, streams, rivers, and lakes. There is a variety of opportunities for recreation in water for individuals, which includes swimming, rafting, canoeing, and surfing.

There are more than fifty state parks and resorts throughout the state of Oklahoma that provides recreation opportunities for residents and tourists. Oklahoma also provides an assortment of different areas for outdoor recreation in the water in the way of river and lakes. There are 500 rivers and streams that span over 5,519 miles in the state of Oklahoma (Wikle, 1991). Oklahoma also has scenic rivers that provide an opportunity for recreation. For instance, the Illinois River receives approximately 350,000 users a year and nearly 2,400 people a weekend floating the river during summer months (Haraughty, 1999).

The lakes in Oklahoma are all man-made. Majority of these lakes are used for recreation, agriculture and municipal water supply. These areas cover one million surface acres with approximately 2,000 miles of shorelines (Wikle, 1991). The National Recreation Commission estimates nearly 18,718,000 visits to recreational lakes in Oklahoma annually.

As stated in Statewide Comprehensive Outdoor Recreation Plan (SCORP) 2007, the demand for outdoor recreation exceeds the available supply in many areas in Oklahoma during the summer months (Caneday *et al*., 2007). The National Recreation Survey and the Environment (NRSE, 2007) states that 13. 3 % of individuals in the United States participated in water-related activities such as swimming, boating, kayaking and surfing. This trend is considered to be similar in the states of Oklahoma (Caneday *et al*., 2007). The majority of the individuals participating in these activities was women (63%) and/or white (79.3%) (NRSE, 2007).

Recreational waters should not contain any contaminant that may cause any type of illness. Primary body contact allows the possibility of ingestion. In Oklahoma, the

primary body contact standard is usually applied during the recreation period of May 1 to September 30. In 2006, the Oklahoma Water Resources board reported that 82 percent of streams in Oklahoma did not meet primary body contact standards (Stubblefield, 2007). The secondary body contacts are not as strict as primary body contact. These guidelines are in effect for the remainder of the year. The secondary guidelines are in place when ingestion of water is not probable. These include such activities as wading, fishing and boating (USEPA, 2004).

In addition, the National Water Quality Inventory (USEPA, 2004) specified that agricultural operations were often the main contributor to fecal pollution in streams. Other common water pollutants include wastewater and storm water runoff. There is an average of 13 outbreaks of waterborne disease annually from recreational waters.

Health Effects

The Centers for Disease Control estimate worldwide that each year 2 million people die from coming into contact with contaminated water. The majority of the deaths are among infants or young children. There is a wide range of syndromes that may occur from coming into contact with contaminated water**.** These may include cholera, dehydrating diarrhea, and abdominal pains. The most common etiologic agents include *salmonella, shigella, E.coli and campylobacter*. There are usually only a small number of outbreaks reported throughout the United States but often they may not be severe (CDC, 2005).

Potential Sources in the Current Study

The city of Stillwater is located in Payne County in Oklahoma. The population of Stillwater is approximately 41,320 people (U.S. Census, 2000). The Stillwater area is diverse in land uses including agricultural, urban, and several areas for recreation. Near Stillwater there is a variety of different types of livestock farming, which includes cattle, swine, and equine. The potential sources of contamination in the Stillwater area would have to come from either human, wildlife or livestock origins.

Objectives of Study

The primary objective of this thesis is to characterize the distribution of indicator bacteria as affected by urban and rural land uses during high and low flow periods. This was accomplished by monitoring two streams in the Stillwater area. This will give managers an idea how potential sources of contamination affect the various alternative indicators. The second objective is to determine the distribution of indicator bacteria between sediments and water column samples.

Generally speaking, regulations are geared more toward monitoring water columns at low flow, but sediments may be resuspended in many cases and cause problems. Statistical analysis was used to determine the significant differences of indicator organisms under different land uses and flow regimes.

2. LITERATURE REVIEW

Water Pollution

The pollution of water can be dangerous and have detrimental effects on people or animals that come into contact with it. Polluted water is water that contains impurity, making the water unsuitable for its intended use (Wright, 2004). Pollution in water includes pathogens, inorganic, and organic pollutants.

Microbial pathogens include bacteria, viruses, protozoa, and other organisms. The origins of these pathogens are from confined animal feeding operation, septic tanks, and sewage discharge. Other pollutants in water are inorganic and organic contaminants (Aull, 2005).

Organic pollutants include materials such as bacteria, insecticides, industrial solvents, and petroleum products. The use of insecticides, pesticides, and herbicides in residential areas also contributes to pollution in streams and other bodies of water (Aull, 2005). Increased nutrient loading in bodies of water leads to euthrophication. Inorganic pollutants usually originate from a natural source. These contaminants include heavy metals, acids, and other chemicals (Troeh *et al*., 2004).

Point Source Pollution

"Point source pollution is any discernible, confined, and discrete conveyance, including but not limited to, any pipe, ditch, channel, tunnel, conduit, well, discrete fissure, container, rolling stock concentrated animal feeding operation (CAFO), landfill

leachate collection system, vessel or other floating craft from which pollutants are or may be discharged. This term does not include return flows from irrigated agriculture or agricultural stormwater runoff" (USEPA, 2004).

Point source pollution refers to pollutants that issue from a pipe or manmade conveyance that can be tracked back to a single source. Point source pollution includes discharge from industrial facilities, publicly owned treatment works and urban runoff (USEPA, 2007a). Since point source pollutants can be tracked back to their origin they can be regulated (Aull, 2005).

Non-point Source Pollution

Non-point source pollution is pollution that is not discharged from pipes or other man-made structures. The problem with non-point source pollution is that there is not one single area that the contaminant comes from. There may be multiple areas in which these pollutants are released. It is difficult to track the source of the pollutant if these substances have traveled a long way before they are discharged into streams or rivers. Since there are no specific points from which these pollutants come, it is difficult to determine who is to blame for the degradation of a certain stream or river.

According to the 2002 national water quality inventory approximately 45 percent of streams were impaired. Forty-seven percent of lakes did not support their designated beneficial uses. The main cause of impairment to these streams and lakes was through runoff from agriculture, industrial and other non-point sources (USEPA, 2007a).

The State of Oklahoma 2004 Water Quality Assessment Integrated report showed the presence of indicator organisms throughout the state of Oklahoma. There was a total

of 5,125 miles of streams and rivers impaired with *Enterococcus*. Streams and rivers impaired with *E. coli* covered 3,333 miles. Fecal coliform have the lowest with 2,699 miles. Potential sources from unknown sources covered 7,361miles of streams and rivers. Agricultural potential sources covered 3,085 miles of streams and rivers (ODEQ, 2004).

National Pollutant Discharge Elimination System (NPDES)

Title IV of the Federal Water Pollution Control Act (PL 92-500), now known as the Clean Water Act, created the system for permitting or regulating the discharge of wastewater; this was known as the National Pollution Discharge Elimination System (NPDES). In this program facilities that discharge pollutants into bodies of water from point sources must obtain permits for the amount of pollution they can discharge. This program has reduced the illicit discharge of pollutants into many bodies of water around the country. As a result, approximately two-thirds of the United States waters are safe for recreational use (USEPA, 2004),(Aull, 2005).

There are two types of NPDES permits, a technology-based limit and a water quality-based limit. Technology-based permits are based on treatment technology employed to reduce contaminants. Water quality based permits are used if technology-based permits provide inadequate protection of various bodies of water (USEPA, 2007b).

Phase I Stormwater

This program relies on the NPDES to regulate stormwater runoff. Phase I was promulgated in 1990 and took effect in 1992 focusing on industrial facilities and Municipal Separate Storm Sewer Systems (MS4s) for cities with a population greater than 100,000. Phase I required these entities to obtain a permit for the discharge of

pollutants. MS4s are defined as a conveyance or system of conveyances (including roads with drainage systems, municipal streets, catch basins, curbs, gutters, ditches, man-made channels, or storm drains). MS4s must be owned and operated by a state, city, town, borough, county, parish, district, association, or other public body. Phase I was designed for collecting stormwater which are not part of public owned treatment works (POTW) (USEPA, 2000).

Phase I requires MS4s and industrial facilities with population greater than 100,000 and construction activities disturbing 5 acres or more to obtain permits.

Phase II Stormwater

The stormwater Phase II was promulgated in 2000 and implemented in 2003 to further improve water quality and aquatic habitats affected by stormwater runoff. Phase II regulates small MS4s and smaller construction areas. These authorities also determine if MS4s in urbanizing areas with populations under 10,000 individuals and population densities greater than 1,000 square miles be included in phase II regulation. These requirements are controlled by the states under their NPDES permitting authority.

The small MS4 management program includes six mandatory control measures. These six control measures are: public education and outreach, public involvement, illicit discharge detection and elimination, construction runoff controls, post construction runoff controls, and pollution prevention (USEPA, 2000).

Phase I and II programs focus on discharge from urbanized areas and construction sites. Such locations contribute high concentrations of pollution due to the amount of impervious surfaces in these areas. Contaminants in urbanized areas include pesticides

fertilizers, animal waste and sediments. Sanitary systems can transport pathogens into stormwater systems through cross connections, combined sewers, and overflows that may cause pathogens to enter streams or rivers. This may cause potential threats to public health, recreation, and the aesthetics to a stream (USEPA, 2000).

Land Uses

Contamination of streams and rivers is widespread in urban and rural environments. The main sources of contaminants are from nonpoint sources (Jeong, 2003). Stormwater runoff carries particulate matter to streams that may have detrimental effects on the stream. The majority of areas in an urban region are covered with impervious surfaces that transport of contaminants efficiently into urban stream and rivers. These surfaces do not allow water to infiltrate into the soil and contribute to ground water. The type of land use and cover has an effect on the transportation of contaminants into streams (Basnyat *et al*., 1999).

Rural Land Uses

Rural areas have been shown to contribute great amounts of pollutants that degrade nearby bodies of water (Graves *et al*., 2002). Agricultural activities such as livestock operations and crop production may be the biggest contributors of pollutant in these bodies of water. Often contaminants from agriculture lands are difficult to track back to their origins. The locality of agricultural land to bodies of water has been found to affect the quality of water (Basnyat *et al*., 1999). Large application of fertilizers and chicken litter have caused elevated nutrient in many streams (Vieux and Moreda, 2003).

The observation of indicator organisms in a watershed may vary depending on the land use. A study performed by Graves *et. al* (2002) monitored fecal coliform and *Enterococci* in a rural stream in Virginia. The area was considered to be a popular place for fishing and swimming. This study found the 37 of 117 samples had fecal coliform exceeding recreational water standards for the state of Virginia. A majority of the samples during the summer and fall exceeded fecal coliform standards for the recreational waters when most people were recreating. Concentration was highest during low flow and warm months (Graves *et al*., 2002). The average density of indicator bacteria during cool seasons and high flows was below the recreational water quality standard. *Enterococci* were also found at high concentration at a majority of the sites sampled during warm weather and low flow. The potential cause of having greater concentration of fecal indicator bacteria was thought to be due to dilution factors pertaining to low levels of water or from the activity of wildlife during warmer months.

Urban Land Uses

A study performed by Jeng *et al*. (2005) on the impact of urban stormwater found high geometric means of *E. coli*, *Enterococci* and fecal coliform in a Lake Pontchartrain estuary. In the areas studied, some recreational activities have been restricted due to pollution from storm water runoff. Samples in this study were taken during dry and wet weather periods. This study found that during two dry periods indicator bacterial densities were elevated. There were also high densities in sediments samples. This study also indicated, during wet periods, indicator bacteria had the highest densities.

Urbanized areas in coastal regions usually have seasonal tourist activity. A study conducted by Reeves *et al*. (2004) had high geometric means of fecal indicator bacteria in dry weather urban runoff. In this study, urban areas were designated as residential, industrial and parks. This study also found fecal indicator bacteria highest in residential areas rather than commercial, agricultural or industrial areas. The main contributor of fecal contamination in these areas may have been from regrowth in sediment or from waterfowl. Over the course of studies all indicator bacteria increased in forebays while the concentration of indicators remained constant at the outlet of wet detention ponds. This study collected samples during baseflow, trace rain, and during rain events. Highest concentration of fecal indicator bacteria was observed during rain fall events. Samples were collected upstream and downstream. There were significant differences between upstream and downstream with respect to total coliform and fecal coliforms. In this study, urban runoff was known to cause a considerable amount of indicator bacteria into bodies of waters (Reeves *et al*., 2004).

The amount of impervious surface has been shown to have adverse effects on the amount of indicators that enters nearby bodies of water. Tufford and Marshall (2002) found the greatest amount of fecal coliform downstream from large commercial areas and mixed urban land uses rather than more rural land uses.

Additional studies have shown increased indicator bacteria in urban areas. A study by Young and Thackston (1999) showed high concentration of E. coli, fecal coliform and Fecal Streptococci in sewered basins in urban areas. This study also found higher concentrations of indicator organisms after rainfall events and lower counts of bacteria during dry periods. Non-sewered areas or areas using septic systems had lower

concentrations of fecal indicator organisms. This study also found higher concentration of these indicator organisms during summer, which corresponds with people recreating. Samples from residential lawns showed high concentrations of fecal bacteria. Highly used or populated areas are more susceptible to these types of contaminants (Young, 1999).

Pathogens Found in Water

There are many different pathogens found in streams that contribute to illness such as viruses, bacteria, and protozoa. Bacteria include a variety of different prokaryotes. All known disease causing bacteria are prokaryotes (Madigan, 2006). Bacteria may be found in the soil or water. They are also found in and on plants and animals. Pathogenic bacterial species include *Salmonella, Escherichia coli O157:H7, Chlamydia, Legionella, Campylobacter, and Yersinia* (Younos *et al*., 2007).

Viruses are the smallest of all pathogens. These organisms are a large group of submicroscopic infectious agents that must have a host to survive. These pathogens are the most durable and require fewer units to infect a host. They may range from 30 to 200 nanometers (nm) in size (Fuhrmann *et al.,* 2005).

Protozoa are unicellular organisms that lack cell walls. They are the largest group of pathogens, including *Giardia* and *Cryptosporidium*. They range from 6 to 100 micrometer (µm) in diameter. These organisms are eukaryotic and may be found in freshwater and marine environments. Many of these species are known to be parasitic in other animals, besides humans. These organisms form cysts that allow them to survive in the environment. Cysts have a chemically and physically resistant coating. They are

found in the soil as well as in the air (Fuhrmann *et al*., 2005). Many of these pathogens are released into the environment at high concentration, and they may be detrimental at very low concentrations.

Survival of Pathogens

There are factors that limit the survival of pathogens in water. Acidic water will cause rapid die-off of most pathogen species, although certain bacteria known as acidophiles are capable of surviving under very acidic conditions. Some pathogens need certain nutrients for growth and survival. These nutrients include organic matter taken by a cell from the environment and used in catabolic or anabolic reactions (Fuhrmann *et a.l*, 2005). Absence of such nutrients will not allow some pathogen to grow. Areas that receive large amounts of sunlight can cause rapid die-off of pathogens.

Pathogens and other fecal contaminants can come from a variety of sources and may survive in many environments. In animal waste pathogens may survive from days to many years depending on the environmental conditions. The release of these pathogens may be through a variety of different pathways such as runoff, infiltration into ground water, and the application of animal waste over cropland. The survival of pathogens in water varies depending on water quality parameters such as turbidity, temperature, oxygen levels, presence of nutrients, pH, organic matter, and solar radiation. As pathogens leave their host into the environment, they begin to adjust and adapt to their surroundings. Some of these organisms can multiply outside the host under suitable conditions. These organisms can also be resistant to antibiotics (USEPA, 2005).

When livestock have access to water bodies, they can contribute a great amount of contamination, and the sediments may become a reservoir for fecal bacteria and pathogens. When stream beds dry out, bacteria become embedded in the sediment. Clay and organic particles may protect the bacteria from unfavorable conditions. Regrowth of some bacteria can occur when the sediment is rewetted. Microbes may also settle and accumulate at the bottom of rivers and lakes. When individuals are recreating, they may resuspend the bacteria and transport the organisms to other areas, become ill or both (Hartel *et. al.*, 2004).

Transport of Bacteria

There are several factors that are important in the transport of microorganisms. These include advection, dispersion, adsorption, and decay or die off. Advection is a process by which microorganisms are transported by the bulk motion of flowing water. Dispersion is the spreading out of microbes through diffusion or turbulence. Adsorption is the removal of bacteria by adhesion to soil particles. Decay-die off is the inactivation of microorganisms due to environmental stresses such as temperature or lack of nutrients (Fallon and Perri, 1996).

Many scientists evaluate the transport of bacteria on two levels, the watershed level and the soil profile level (Coyne *et. al*., 2001). The watershed scale is much larger than the soil profile scale, as bacteria travel over a large area before they reach their final destination.

The soil profile scale looks at how the microbes move through the soil profile. Saturated flow is a significant factor in the transportation of bacteria through soil pores.

The rapid movement of fecal bacteria through the soil can cause contamination of ground waters. The topography of a given area has a great effect on the movement of bacteria through the soil column as well as the watershed (Coyne, 2001).

Review of Indicators

Testing a body of water for certain pathogens directly is expensive, dangerous, and complicated. Instead of monitoring pathogens in water samples, researchers and environmental managers normally use indicator organisms as surrogates for pathogens in water samples. These organisms are more easily measured than pathogens. The indicators most commonly used are *Enterococci, E. coli* and fecal coliform. If there are high concentrations of any of these organisms in recreational water, there is a reason to suspect fecal contamination. This poses a threat to swimmers and others who come in contact with contaminated water. Bacterial indicators may remain in a stream at levels that are above the EPA standards even after a heavy rain. Fecal bacteria are derived from the human and animal intestine, where they help in the digestive process. These organisms are used as indicators because they are found in large numbers in human and warm-blooded animal feces. Often, using one indicator organism can be misleading. There are certain criteria for indicator organisms which include (Bitton, 2005):

1. Resistance to environmental factors similar to pathogens;
2. Should not multiply in the environment and also be non-pathogenic;
3. Should be easy to detect in rapid and inexpensive ways;
4. Member of the intestinal microflora in humans and warm blooded animals;

5. Presence in high number in fecal matter.

Fecal Coliform Bacteria

These organisms are thermotolerant bacteria that can ferment lactose at 44 °C. These are facultative aerobic gram positive organism, rod shaped, non-spore forming bacteria. They are enumerated in two ways, multiple tube fermentation technique and the membrane filtration technique (Bitton, 2005). The organisms are often not reliable in indicating viruses and protozoa.

Fecal coliforms live in the intestines of warm blooded animals such as human, domesticated animals and wildlife and are found in feces. These bacteria generally are not harmful, but they are indicators of fecal contamination that may also include certain pathogens. Some of these diseases include typhoid, dysentery, hepatitis A, and cholera.

Escherichia coli (E. coli)

Escherichia coli is a subgroup of fecal coliform and the most common of the group. Like other fecal coliform, *E. coli* is aerobic and facultative anaerobic, gram negative, and non-spore forming. They usually are rod-shaped, and they ferment lactose with gas production. These organisms are also thermotolerant. In the gastrointestinal tract, these organisms aid in the processing of vitamin K (Madigan, 2006). E. coli is found in the intestines of birds and mammals. It is generally not found in groundwater or streams and rivers unless contaminated by fecal matter. Membrane filtration is one of the methods used to enumerate *E. coli* from the natural environment. There are some *E. coli* that are toxic and known to cause gastrointestinal illnesses. The presence of *E. coli* is

considered a better indicator of risk to bather in recreational waters than other fecal coliforms (Leecaster *et al*., 2003).

Enterococci

Enterococcus is a subgroup of the fecal streptococci bacteria. The organisms are gram positive and facultative anaerobic. Although *Enterococci* are present in the feces of animal and human, they are more human specific than *E .coli* or fecal coliform. There are two types of *Enterococci* which may cause disease within humans, the *Enterococcus faecalis* and *Enterococcus faecium*. These bacteria are also the most resistant to antibiotics (ODEQ, 2006). They are used as indicators in marine waters, because they have the ability to survive in salty water, at 6.5 % NaCl, high temperature and high pH. These organisms generally survive longer in the environment than other the indicator bacteria (Bitton, 2005). They can also persist and regrow under many different environmental conditions. Clay particles protect *Enterococci* in the soil during adverse conditions (Hartel *et. a.l*, 2004). These organisms can be detected by using membrane filtration technique (Dufour *et al*., 1981) or the Enterolert method (IDEXX, 2007).

Microbial Source Tracking Techniques

A way of managing water quality in streams when it is attributed to microbes is by tracking the source of contamination. Tracking methods allows the manager to find the pollutant at its origin and control it at the source. When microbial source tracking is used to determine the source of fecal bacteria in the environment, it is called Bacterial Source Tracking (Marshall University forensic science center, 2005).

Also, bacterial source tracking improves modeling and helps scientist get a better understanding of the fate of certain bacteria. “These bacteria can be tracked back to urban or farming sources. The method for BST uses bacteria uniquely found in human and animal excrement” (Marshall University forensic science center, 2005).

Techniques used to ID bacteria to track back their origin include ribotyping, pulse-field gel electrophoresis (PFGE), denaturing-gradient gel electrophoresis (DGGE), and antibiotic resistance analysis (ARA). Ribotyping, also known as molecular finger printing is a method of identifying microbes by analyzing DNA fragments produced from restricted enzyme digestion of genes encoding in the 16s rRNA. This method provides a fingerprint of the bacterial genome. The DNA for certain bacteria will produce patterns that are unique. This method may be effective in discriminating between human and animal sources, but it is a very expensive method compared to some of the other types of tracking methods (Meays *et al*., 2004).

Pulse-field gel electrophoresis is a fingerprinting technique that uses enzymes that are rare on the entire DNA genome. This genome is separated by subjecting it to electrical pulses (Meays *et al*., 2004).

Denaturing-gradient gel electrophoresis is another technique by which the genes are separated into segments that are similar in size but different in base sequence. The analysis is based on melting properties of the amplified DNA sequences. Finally, antibiotic resistance analysis is a method to detect those bacteria from human and animal sources. The basis is that human fecal bacteria will have greater resistance to certain antibiotics, while livestock will have a greater resistance to other antibiotics. This method

is successful because humans and animals are generally exposed to different types of antibiotics (Meays *et al*., 2004).

Other Techniques Relating to the Detection of Fecal Contamination

There are also other methods to differentiate between human and non-human sources of fecal contamination in streams and rivers. Quantifying bacteria from a given source is one of the focal points of this investigation. These methods include using bacteriophages, Fecal Coliform to Fecal Streptococci Ratio (FC/FS ratio), detergents/ optical brighteners, and caffeine. Bacteriophages may be used to determine the source of fecal contamination. They also may be poor indicators of contamination from human sources, but this method is a useful indicator of domestic farm animals. Much more research is needed in this area.

The Fecal Coliform to Fecal Streptococci Ratio (FC/ FS) is an inexpensive method. A ratio of four or greater is considered to be indicative of a human source. A ratio of less than 0.7 is thought to indicate a non-human source. There is a major weakness of the FC/FS ratio in that it does not take into account the die off rate of each type of bacteria. Different die off rates can change the ratio (Sargent, 1999).

Optical brighteners are chemicals that have a high affinity for cotton. When these are exposed to UV light, they emit a blue fluorescence. Optical brighteners have been used as indicators of septic tank or sewage discharge. This method is useful within a watershed, but there can be high variation in natural background fluorescence between watersheds.

Caffeine detection has also been suggested as an indicator of human fecal sources. Caffeine has been detected in close proximity to combined sewer overflows that have been discharged. Caffeine levels must be high in order to quantify the amount of contamination (Sargent, 1999).

Examples of Outbreaks of Water-borne Disease

The introduction of microbial pollution has caused an outbreak of disease in many drinking and recreational waters. In 1998 nearly 729 beaches were closed due to high level of bacteria (Rose and Grimes, 2001). EPA recommends that states use Enterococci and E. coli as a criterion with illness no greater than 14 illnesses per 1000 swimmers for fresh water and no greater than 19 illnesses per 1000 swimmers for salt water (USEPA, 2002a). There have been many incidents where fecal contamination from wildlife was the primary source.

Furthermore, more than100 million Americans rely on groundwater as their primary source of drinking water, that are not disinfected before they are used. In areas where livestock production is concentrated, many of these wells may get contaminated with fecal bacteria. If there are extreme amounts of nutrient within a stream or river, there probably will be bacteria problems in that stream (USEPA, 2002a).

According to the Ambient Water Quality Criteria for Bacteria, in 1998 in Alpine, Wyoming, nearly 157 people were infected from contaminated water supplies. In Milwaukee, there was a Cryptosporidium outbreak which resulted in 403,000 illnesses and roughly 100 deaths (USEPA, 2002a). The source of Cryptosporidium was suspected to be agriculture runoff from neighboring dairy farms. The Environmental Protection

Agency recommends that states adopt water quality criteria for bacteria in water bodies that designated primary contact recreation in order to ensure protection of human health (USEPA, 2002a).

Effect of Fecal Contamination on Coastal Beaches

Fecal contamination poses a great threat for beach goers. In 2001, 13,410 beaches were closed due to poor coastal water quality near beaches in the United States (McLellan, 2003). This was due to the fact that indicator organisms were greater than standards set for primary contact by the United States Environmental Protection Agency. On the other hand, these indicators do not always correspond to the presence of pathogen within a body of water.

In coastal areas, contaminants may come from a variety of sources many of which are difficult to determine. Humans may contribute large amounts of contamination from sources such as sewage overflow and improper sewage systems. Impervious surfaces such as streets, parking lots and buildings have caused these indicator organisms to run off into the coastal areas. This contributes to high levels of fecal contamination in coastal areas around the country. Wildlife and livestock can contribute to contamination in coastal areas as well. Birds that are near coastal area can contribute large quantities of contamination, especially when these birds are in large flocks. Sand in coastal areas can shelter fecal bacteria that have runoff from human sources or deposited by wildlife. This is a very serious issue considering the number of people who visit and recreate at beaches every year (McLellan, 2003).

Fecal contamination is a problem in other countries as well as the United States. Two urban beaches in Proto, a city in Portugal, were monitored for contamination in 2001 (Bordalo, 2003). This is an area that tourists visit frequently and water quality is important to the tourism and the economy of this area. These areas were sampled for 18 days consecutively collecting samples three times a day. Due to sub-standard sewage systems, this area has high fecal contamination in the coastal zones, which is where the majority of the population of Proto lives. After analysis of the data, researchers found fecal coliform to be abundant in both beaches. The average amount of contamination was well above European Union standards. This study also found that more contamination was found during the early morning, with the number of contaminants dropping during the afternoon. This also showed that the time of the day may affect the amount of fecal contamination in the water due to solar radiation, pH and temperature. This further displays the need for managers across the world to combat the problem of fecal contamination in the world water (Bordalo, 2003).

Reducing Contaminants in Water

In order for managers to reduce fecal contamination of water bodies, watershed managers must first identify the potential sources of contamination. Implementing best management practices can greatly reduce the amount of fecal contamination in streams and rivers (USEPA, 2005). There are many methods that may be used to help reduce fecal bacteria in streams and rivers. One way involves using a vegetative buffer along rivers and streams. This helps to filter the surface-runoff that may contain fecal bacteria. Septic systems are in compliance and functioning properly are important because

effluents from septic tanks contain a variety of substances including bacteria. Improperly functioning septic systems allow wastewater to leach directly into the groundwater or rise to the surface (Haraughty, 1999).

Another way to prevent livestock or domestic animals from having access to streams is by providing barriers or fences (Collins, 2003). However, wildlife can also be a big problem when trying to reduce fecal contamination from stream and rivers. If best management practices are implemented to reduce human and domesticated animal, then wildlife may contribute most of the contamination to a stream. Establishing a riparian zone may help reduce contaminants in streams, but could increase contribution from wildlife by providing habitat near the stream. This may be difficult to estimate (Haraughty, 1999).

Literature Review Conclusion

This literature review shows that pathogens are a significant cause of impairment in rivers and streams. In urban and rural areas, runoff is the primary sources of these pathogens. The aim of this study was to aid in selecting the best indicator(s) for control of pathogens. Monitoring streams and river for pathogens or fecal contamination can be difficult and expensive. Fecal indicator bacteria are used for detection of potentially infectious pathogens in water because they are known to be high in number in animal and human feces. The indicator organisms monitored in this study were *E. coli*, *Enterococci*, and fecal coliform which are the most commonly used indicators.

This paper has reviewed the literature on the impacts and the potential threats of contamination. This literature review concludes that monitoring indicator species is

important for the health of the public. The findings suggest that this issue affect urban and rural areas which are monitored in this study. The material gives a better understanding of methods and processes that we may used to determine the presence of pathogen.

Research for this thesis addresses the distribution and occurrence of indicator bacteria associated with different land uses, flow regimes and sediment vs. water column because these parameters can affect the concentrations of contaminant in bodies of water. The following chapter describes the methods use to meet this purpose.

There are a number of techniques available for enumerating the presence of fecal contamination in water (as discussed in the literature review), and it is important to develop low-cost simple methods to monitor them. The focal point is the selection of the best indicator(s) for protection of public health and safety of individuals participating in water related activities. Often one indicator may violate standards while another does not. Additional research is needed in this area to find the best indicator to evaluate the risk of pathogens in a body of water. There is also more research needed to find less expensive ways to track these bacteria back to their sources.

3. METHODS

Overview

Bacteriological tests in water are used to evaluate the quality of water and determine the potential health risk from waterborne diseases. In this study, multiple indicators including, *Enterococci*, *E. coli* and fecal coliform tests, were used to evaluate or characterize contamination in the Stillwater area. These are the most commonly used indicators of bacterial contamination. The land uses were also determined to distinguish between urban and rural and differences in their impact.

Sampling Sites

Samples were taken from four locations in the Stillwater Creek watershed during the months of June 2007 through August 2007. Sampling sites includes Boomer Creek, and Cow Creek. Figure 3-1 is an illustration of sampling locations.

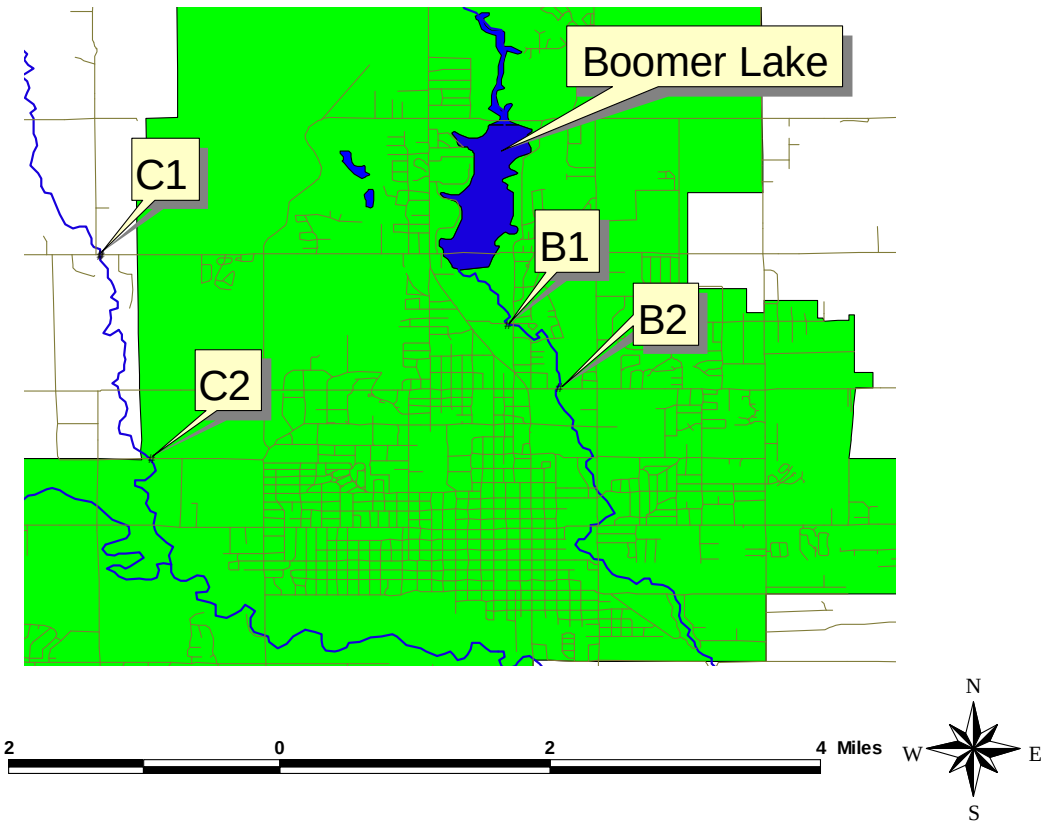

Figure 3-1: Sampling Sites on Boomer and Cow Creeks. B1 and B2 are upstream and downstream of urban residential areas. C1 and C2 are upstream and downstream of a rural agricultural area.

Sites Description

Sites located on Cow Creek were in heavily wooded areas. The first site, C1 is located on Cow Creek at Lakeview Road just outside of Stillwater City limits. There are a few residential homes located upstream from this site. The majority of these homes use septic systems. The area consists of farmland and pasture land. Figure 3-2 is a photograph of the sampling location. The second location, C2 (Figure 3-3) is downstream at Virginia St above a concrete crossing. Between C1 and C2 are Oklahoma State University Swine facilities, Equine center, and dairy farm. The downstream location

is surrounded by pasture land as well as crop lands. There are livestock facilities approximately 20 yards from the sampling location (Figure 3-4). Cow Creek is surrounded by a majority of agricultural lands. All sites were located near bridges for greater accessibility.

Figure 3-2: Cow Creek Upstream Site, C1

Figure 3-3: Cow Creek Downstream Site, C2

Figure 3-4: Swine Facilities just above downstream location

Boomer Creek flows directly through the city of Stillwater. The creek is fed directly from Boomer Lake. The first site, B1 is located on Boomer Creek just downstream from Boomer Lake at Franklin St (Figure **3-5**). This site is adjacent to several commercial businesses. The fourth site, B2 is located on Boomer Creek downstream, after it passes through a residential area (1 Figure3-6). It is also downstream from apartment buildings. Boomer Creek is predominantly surrounded by residential housing.

Figure 3-5: Boomer Creek Upstream Site, B1

Figure3-6: Boomer Creek Downstream Site, B2

Samples were taken first from the sites that were upstream, then downstream. All samples were taken as grab samples in sterile bottles facing upstream. At each location sediment and water column samples were taken separately and labeled appropriately.

Sediment samples were collected as grab samples from the streambed using sterile 150 ml nalgene bottles. The covered bottles were lowered into the bottom of the stream, tops were removed to scoop sediment-water samples. There were duplicates taken at each location during sampling. Later samples were taken back to the laboratory

and diluted at 10^{-3} using a graduated 10 ml pipette to transfer the sediment-water mixture. Samples were processed using the IDEXX and EPA method 1604 (USEPA, 2002b).

High-flow samples were taken three days after a storm event during a wet weather period. Low-flow samples were taken at least a week after a rain event. The samples were taken back to the laboratory and processed within 6 hrs after collection. There were a total of 64 samples taken during the study period. The rainfall record for the period is shown in Appendix B.

Water Quality Parameter Methods

pH was determined by using a Denver Instrument electronic pH meter in the laboratory . Turbidity was measured in the laboratory using a Beckman DU 640B Spectrophotometer as absorbance of samples at 595 nanometer (nm) using deionized water as a blank. Temperatures were recorded at each site upon collection of sample.

Method for Bacterial Indicators

Enterococci

Enterococci were detected using the Enterolert method (IDEXX, 2007). "Enterolert uses a defined substrate technology (DST) nutrient indicator to detect *Enterococci*. This method has been used successfully in testing marine and fresh water samples. The nutrient indicator fluoresces when metabolized by *Enterococci*. DST improves accuracy and avoids the need for hazardous sodium azide suppressants used in

traditional media" (IDEXX, 2007). This method provides a Most Probable Number based on the number of wells showing presence of *Enterococci*.

Results for Enterolert were obtained by collecting 100 ml water samples using IDEXX 100 ml nalgene bottles. Water column samples were diluted 10^{-1} and sediment samples were diluted at a 10^{-3} V/V ratio. One package of powdered Enterolert reagents was added to each sample. The samples were shaken vigorously and then poured into Quanti-tray 2000. These trays were then sealed automatically using a Quanti-tray sealer model 2X from Idexx. Trays were incubated for 24 h at 41° C ± 0.5. Results were measured by placing quanti-trays under long wave ultraviolet light. Wells that fluoresced under UV light were known to be positive. Positive wells were counted and recorded. Quanti-trays showing no florescence are considered to be negative for *Enterococci*. The number of large and small positive wells were counted and recorded. Most Portable Numbers were determined by referring to the table provide by Idexx Laboratories.

Enterococci were confirmed by plating aliquots from positive wells. The backs of quanti-trays with positive wells were sterilized using 90% ethanol. Sterile pipette tips were used to pierce the back of each positive well. A 10 µl aliquot of each positive well was streaked on plates containing enterococcosel agar. These plates were incubated for 48 h at 37º C. After 48 h, plates were examined for colonies surrounded by black halos, which were considered to be confirmed as *Enterococci*. The plates containing black color were counted and recorded (Appendix B).

E. coli and Fecal Coliform

Escherichia coli and fecal coliform were processed using the membrane filtration technique. Sterile 0.45 µm membrane filters were placed on a nalgene 115 ml filter unit. Ten ml of sterile water was added into the funnel followed by 100 ml of the sample. The vacuum was turned on until all the water had run through the filter. Using flamed forceps, the filters were gently placed on MI agar plates. Filters were slid across the agar in a rolling action to avoid air bubbles between filter and agar. Agar plates were placed upside down in a Precision Economy incubator at 35 ° C for 24 h, according to the EPA Method 1604 (USEPA, 2002). After 24 h plates were exposed to long-wave ultraviolet light. Colonies that fluoresced were counted as E. coli. Other colonies found on the plates were considered to be non-*E. coli* fecal coliform. Plates were counted and recorded. The total number of colonies, fluorescent and non-fluorescent was recorded as total fecal coliform.

Data Analysis

The amount of enterococci in each sample was quantified by using the most probable number technique. This technique uses a series of dilutions of a natural water sample to determine the highest dilution yielding growth (Madigan, 2006). This resulted in positive and negative isolates from each quanti-tray. This is used to get an estimate of the population present in each sample. The number of positive isolates from quanti-tray was compared to the total number of isolates streaked on enterococcosel agar.

Enumeration of *E. coli* and fecal coliform was done by using the direct plate count method (Madigan, 2006). Plates were counted for colonies. The ideal plate number is between 20-80 colonies. Plates with more than 80 colonies were divided into quarters.

One section of the plate was counted, and the total was multiplied by four to get an estimate of bacteria on the plate. The final calculation used the formula recommended by EPA method 1604.

Statistical Analysis

The geometric means of sediment and water column samples were calculated to compare indicator organisms under different land use and flow regimes. The geometric mean calculates the nth root of the product of n samples. The geometric mean tends to reduce the effect of very low or high values in a given sample size. This procedure involves a log transformation of the data collected (Freund, 2003). The geometric mean was calculated using Microsoft Excel version or SAS.

Analyses of Variance (ANOVA) were done to determine the differences between the geometric means of indicator species as affected by land use, flow regimes, sample types (sediment vs. water column), and location. This method tests the equality for a given set of means in a data set to evaluate statistical significance (SAS).

The ANOVA was performed on log transformed data to compare the interaction of the sample type, land uses, stations and flow. The interactions are differences or inconsistencies of the main effect response for one factor across the levels of one or more of the other factors. This model is added when one or more variables depend on other variables (Freund 2003). This analysis was done to compare the effect of land uses, stations, and flow on the indicator organisms. In interaction, if we reject the null hypotheses, we compare factors at each and every level. If we do not reject the null hypothesis we look at the test for the main effects.

Statistical significance for interactions between different conditions such as sample type, land uses, station location, and flow was inferred for P=0.05. A non-significance level would be P> 0.05. If the null hypothesis was rejected then the analysis is considered to be statistically significant.

Correlation analysis was used to determine the relationship between indicator organisms. The correlation was performed using SAS, which provided an r-value. A p-value of 0.05 was used to determine statistical significant correlations.

4. RESULTS

The samples were collected at four dates over a two month period from, June 12 to August 6, to determine microbiological water quality, and the effect of different land uses on two streams, Boomer and Cow creeks, in the Stillwater, Oklahoma area. Analysis of indicator bacteria was performed on samples from sediment and water column, as well as taking samples from low flow and high flow on two adjacent streams representing urban and rural land uses. Downstream stations had more of the designated land use than upstream stations. The first two sampling dates, high flow samples had nearly 0.5 inches of rainfall in the previous three days. The final two sampling dates, low flow samples received no rain in the previous week. The rainfall record is shown in the Appendix C.

Water Quality Parameter Analysis

Cow Creek

Table 4-1 lists the physical and chemical characteristics of the water samples obtained from Cow Creek during the study period. Cow Creek temperatures were relatively constant during the months sampled from, June to August. Temperature ranged from 21° C to 25° C. As the summer progressed, stream temperature rose. The pH was near neutral during sampling periods. There was little difference among pH value

station to station or date to date (Table 4-1). High and low turbidity measurements were observed at all locations throughout the sampling process. During high flow the turbidity of samples was lower than samples taken during low flow. The turbidity of samples was also greater upstream than in downstream samples. As shown in Table 4-1, Cow Creek upstream samples displayed greater turbidity levels than downstream samples in June and July during high flow sampling events. The low flow samples in July and August also had higher turbidity upstream. The highest turbidity was observed upstream with a value of 1.98 under low flow conditions on August 6, 2007. The turbidity downstream on that date was 0.354.

Table 4-1: Cow Creek Water Quality parameters

Stations	Date	Temp ° C	pH	Turbidity (O.D.)	Flow Regimes
Cow Upstream	6/12/07	21	7.75	0.406	
Cow Downstream	6/12/07	21	7.64	0.209	High
Cow Upstream	7/9/07	22	7.79	0.025	Flow
Cow Downstream	7/9/07	24	7.59	0.0242	
Cow Upstream	7/22/07	25	7.75	1.82	
Cow Downstream	7/22/07	25	7.62	0.399	Low
Cow Upstream	8/6/07	22	7.65	1.98	Flow
Cow Downstream	8/6/07	25	7.87	.354	

Boomer Creek

Temperatures in Boomer Creek were similar to temperatures in Cow Creek. The pH values ranged from 7.12 to 8.02 (Table 4-2). For the most part, there were not large differences in pH values. Boomer Creek had consistently lower turbidity during high flow than in low flow. There were few samples with high levels of turbidity. Samples downstream on July 22, 2007 indicated high levels of turbidity with a value of 1.37

(Table 4-2). The highest values were observed on August 6, 2007. The upstream values were 1.96 and downstream were values similar with 1.95 (Table 4-2).

Table 4-2: Boomer Creek Water Quality Parameter

Stations	Date	Temp ° C	pH	Turbidity (O.D)	Flow Regimes
Boomer Upstream	6/12/07	22	7.5	0.0627	
Boomer Downstream	6/12/07	21	7.12	0.0657	High Flow
Boomer Upstream	7/9/07	25	7.96	0.236	
Boomer Downstream	7/9/07	25	7.84	0.0458	
Boomer Upstream	7/22/07	26	8.02	0.322	
Boomer Downstream	7/22/07	24	7.75	1.37	Low Flow
Boomer Upstream	8/6/07	25	7.58	1.96	
Boomer Downstream	8/6/07	23	7.95	1.95	

Microbial Indicator Organisms

The three microbial indicators evaluated were fecal coliform, *E. coli* and *Enterococci*. Results for high flow are shown in Table 4-3. Cow Creek and Boomer Creek showed high levels of microbial indicators at each location during high flow. There were usually higher levels of indicators found downstream than upstream at each stream. The sediment sample had high concentration for all indicator organisms at most observations. Many water column samples exceeded the single sample water quality standards.

Boomer Creek High Flow

High flow represents storm water discharge into Boomer Creek. Microbiological water quality on Boomer Creek was poor during high flow. All indicator organisms had higher geometric means upstream in the water column than in the sediments.

Downstream, *E. coli* and fecal coliform had greater geometric means in the sediment while *Enterococci* were greater in the water column.

Escherichia coli and fecal coliform had higher geometric mean upstream in the water column. *Enterococci* had higher geometric means downstream in the water column. All indicator organisms had higher geometric means downstream in the sediments. Both upstream and downstream samples in the water column for indicator organisms exceeded water quality standards.

Cow Creek High Flow

On Cow Creek high flow, both *E. coli* and fecal coliform concentrations were higher in sediment samples than in the water column. Enterococci were found to have higher geometric means in the water in both the upstream and downstream locations. All indicator organisms had higher geometric means downstream than in upstream samples (Table 4-3) Downstream *E. coli* exceeded the USEPA recommended standards. The geometric mean for fecal coliform in the water column met water quality standard. *Enterococci* in the water column for both downstream and upstream exceeded the recommended standards (Table 4-3).

Table 4-3:Geometric Means of Indicator Bacteria during High Flow(2 replicates, 2 dates)

Stations	E. coli		Fecal Coliform		Enterococci	
	Water column	Sediment	Water column	Sediment	Water column	Sediment
Boomer Up	1630	118	697	204	108	21
Boomer Down	370	22400	240	1760	145	46
Cow Up	52	3447	86	92	92	33
Cow Down	830	48440	180	9360	460	124

* Units in CFU
*fecal coliform excludes *E. coli*

Boomer Creek vs. Cow Creek High Flow (Urban vs. Rural)

Escherichia coli geometric means were higher at Boomer Creek in the water column upstream under high-flow conditions, while Cow Creek was higher downstream. In sediment samples, Cow Creek had higher geometric means than in Boomer Creek.

As shown in Table 4-3, Boomer Creek in the water column had higher geometric means for fecal coliform than in Cow Creek. Sediment samples had higher concentrations upstream on Boomer Creek, but Cow Creek had higher concentrations downstream (Table 4-3).

In the case of *Enterococci* in the water column, upstream geometric means were higher at Boomer, while the geometric means were higher downstream on Cow Creek. In the sediments, the geometric means were higher on Cow Creek (Table 4-3).

Boomer Creek Low Flow

Geometric means of indicator bacteria in the water column during low flow were lower than corresponding samples taken during high flow. Sediment samples for both *E. coli* and fecal coliform were greater than water column samples. *Enterococci*, however, were slightly higher in the water column than in the sediments.

Escherichia coli and fecal coliform had higher concentrations downstream in the water column than upstream. On the other hand, sediment samples were higher upstream. *Enterococci* had similar values in water column, but the downstream location was higher in the sediments. As shown in Table 4-4, most water column samples met water quality standard with the exception of *E. coli* downstream.

Cow Creek Low Flow

In Cow Creek, indicator organisms were also lower during low flow. Fecal coliform and *E. coli* had higher geometric means in the sediments than in the water column. *Enterococci* had higher geometric mean in the sediments upstream and similar values downstream (Table 4-4).

In the water column, *E. coli* were higher geometric means upstream, but sediment samples were higher downstream. Fecal coliforms were higher in both the downstream. *Enterococci* had higher geometric means downstream in the water column but in the sediment there were higher concentrations upstream.

Escherichia coli exceeded water quality standard upstream in the water column, as shown in Table 4-4. Fecal coliforms met water quality standard. *Enterococci* violated the U.S. EPA recommended standard downstream in the water column.

Boomer Creek vs. Cow Creek Low Flow

In low-flow samples for *E. coli*, Boomer Creek had higher geometric mean downstream in the water column than Cow Creek (Table 4-4). As shown in Table 4-4, upstream Cow Creek geometric means were slightly greater than Boomer Creek upstream. In the sediment, upstream samples were similar at each location. The sediment downstream had higher geometric mean in Cow Creek (Table 4-4).

Fecal coliform had higher geometric means on Boomer Creek upstream in the water column. Downstream, the geometric means for Cow Creek was slightly greater than Boomer Creek (Table 4-4). Fecal coliform geometric means for sediments were

greater downstream in Boomer Creek than in Cow Creek. *Enterococci* had greater concentration in both the water column and sediment on Cow Creek (Table 4-4).

Table 4-4: Geometric mean of Indicator Bacteria during Low Flow (2 replicates, 2 dates)

Stations	E. coli		Fecal Coliform		Enterococci	
	Water column	Sediment	Water column	Sediment	Water column	Sediment
Boomer Up	124	24490	58	4920	24	15
Boomer Down	239	21245	28	14230	24	23
Cow Up	166	23830	40	10020	32	96
Cow Down	113	91640	42	10590	62	60

* Units in CFU
*fecal coliform excludes *E. coli*

Comparison between Indicators during High and Low Flow

The data described patterns within the individual creeks with the lowest geometric mean of indicator bacteria during low flow and highest during high flow (Table 4-3)(Table 4-4). Results from high flow events indicated increased concentrations of indicator organisms. The geometric means of *E. coli* were higher concentration during high flows at each location except for Cow Creek upstream. However, low flow geometric means showed excessive amount of *E. coli*. As shown in Table 4-3 and Table 4-4, fecal coliform and *Enterococci* were greatly affected by the amount of precipitation. During low flow both indicator organisms were below the U.S. EPA recommended standard in most cases. *Escherichia coli* was noticeably higher than other indicators during low flow (Table 4-4). During high flow, *E. coli* were once again more prevalent than other indicator bacteria (Table 4-4). Indicator organisms were consistently detected in sediment sample during low and high flows. High flow samples exhibited poor microbiological water quality for all indicator organisms at both creeks. There was a connection between stream flow and the amount of indicator bacteria present in streams

5. DISCUSSION

Overview

The research for this thesis focuses on the distribution and occurrence of indicator organisms in two streams. This section provides discussion of the results shown in the preceding chapter. The conclusions are derived from statistical analysis of land uses, flow regimes, and sample types.

Correlations

Correlations were analyzed (Table 5-1) to determine the relationships among the indicator organisms total fecal coliform (*E. coli* and fecal coliform), *E. coli*, fecal coliform, and *Enterococci*. The correlation between *E. coli* and total fecal coliform had an r of 0.941 with a p-value of <.0001 which is highly significant. This strong correlation demonstrates that *E. coli* may be used as the indicator because these organisms consistently predict each other. The correlation of fecal coliform to total fecal coliform was also highly significant with p-value of <.0001 and an r of 0.677. These results are expected since both fecal coliform and *E. coli* are measurements and subgroups of total fecal coliform. Total fecal coliform is a good predicator of each of these organisms. The correlation between *E. coli* and fecal coliform was significant (r=0.387, p=0.0285) as well. This correlation is also projected because *E. coli* is a subgroup of fecal coliform. On the other hand, *Enterococcus* is not significantly correlated with any of the coliforms, *E coli*, fecal coliform or Total fecal coliform. These correlations show that neither of these

organisms was able to predict *Enterococci*. These correlations may suggest that multiple indicator may be important when monitoring for pathogens.

Table 5-1: Pearson Correlation Coefficients, N=32 Prob > |r| under H0: Rho=0

	E. coli	Fecal coliform	*Enterococci*	Total fecal coliform
E. coli	1.00000			
Fecal Coliform	0.38734 0.0285	1.00000		
Enterococci	0.27148 0.1328	0.12660 0.4899	1.00000	
Total fecal coliform	0.94055 <.0001	0.67697 <.0001	0.26478 0.1430	1.00000

*fecal coliform excludes *E. coli*

Analysis of Variance

Geometric means were compared thru ANOVA. Means were considered to be statistically significant at approximately $p \leq 0.05$. Comparison of means with p-values near, but higher than 0.05 were reported with their p-value and considered marginally significant. The effects and interactions of variables for sample type (water column or sediment), flow (high or low flow), land use (urban or rural), and location (upstream or downstream) were examined for each bacterial indicator, Total fecal coliform, Fecal coliform, *E. coli, and Enterococci*.

E. coli was the only indicator even marginally significant for four-way interaction (p=.0534.) This interaction suggests that the concentration of *E. coli* was affected by one or more variables, specifically sample type, flow, land use, and stream location.

Further analyses tested effect slices in which each test variable was fixed. Effect slices show within which measurement occasions there were differences between

experimental groups. The test of effect slices for stream location (upstream vs. downstream) weren't significant at any level. The effect of sample type (sediment vs. water column) was statistically significant at all levels (Figure 5-1) with sediments always significantly higher than water column.

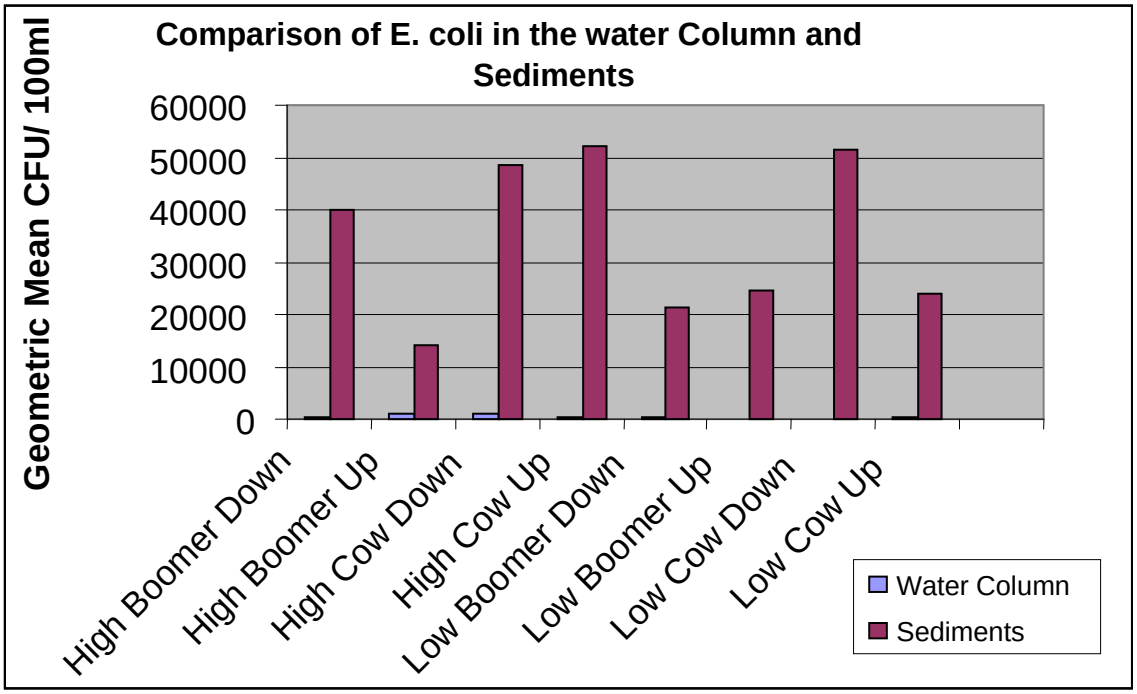

Figure 5-1: *E. coli* Water Column vs. Sediments at Boomer and Cow Creeks, up and downstream stations, under high and low flow regimes.

Fecal coliform four-way interactions, three-way interactions, and two-way interactions weren't significant. Only the main effect of sample type (Sediment vs. Water column) was significant with p-values of <.0001(Figure 5-2). Other main effects such as location, land use and flow regime weren't significant.

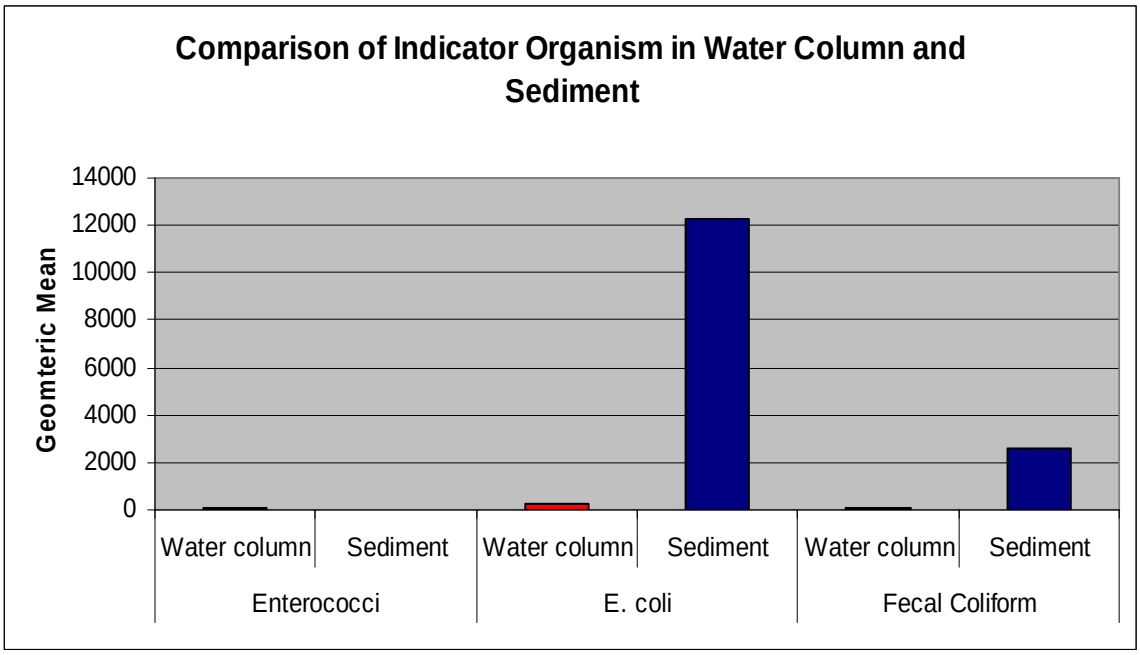

Figure 5-2: Indicator Organism Water Column vs. Sediments (fecal coliform excludes E. coli)

The four-way interaction and three-way interaction weren't significant (Appendix D). The two-way interactions for enterococci weren't significant, as well. This says that the other variables didn't have significant, consistent, effects on the concentration of *Enterococci*. There were no significant interactions and the other main effects weren't statistically significant.

Sample Types (Sediment vs. Water column)

Sediment and water column samples taken at both creeks showed the presence of fecal indicator bacteria. Sample type plays an important role in the concentration of indicator bacteria in a given sample. Quantifying the bacterial concentrations between the different samples types allow a better understanding of the occurrence and distribution of indicator bacteria.

The bacteria in sediment samples were noticeably higher than water column samples with the exception of *Enterococci* during high flow in the unprocessed data. This was expected due to the fact that sediment settles to the bottom of stream beds and bacteria may accumulate in the sediment where they may survive longer (Hartel *et al*., 2004). There are no standards for the maximum acceptable concentrations of indicator organisms in sediment.

Water column samples were generally lower than sediment samples. Bacteria found in the water column could be resuspended from the sediments or bacteria in the water column could be underestimated because the bacteria could fall out of suspension (Davis and Barr, 2006). Water column sampling alone may not accurately reflect the presence of bacteria in the sediments.

In some cases, geometric means were high for all indicator organisms in both the sediments and water column with respect to standards. For instance, *Enterococcus* was found to have high geometric means in the water column indicating the likely presence of human fecal matter. *Enterococcus* was also less in sediment than in water column on both creeks. There was not a significant difference between the water column and sediments. As indicated previously, *E. coli* and fecal coliform were significant for sample types. In most cases, the concentrations of E. coli and fecal coliform were well above recommended standards for both water column and sediment samples. The high concentrations of indicator bacteria in both the water column and sediment during low flow suggest that the source of bacteria does not rely on precipitation events to enter into the streams. The potential sources may be from failing septic systems, septic leachate, or animals in the creek.

Flow Regimes

The study supported the hypothesis that precipitation had an effect on the concentration of indicator organisms present in the streams. *E. coli* varied significantly with flow in the water column at Boomer Creek upstream and Cow Creek downstream, with p-value of .0351 and .0444 respectively (Figure 5-3). The test of flow for fecal coliform was highly significant with p-values of <0.0001. The test of flow for Enterococci were significant with p-value of 0.04 (Figure 5-4).

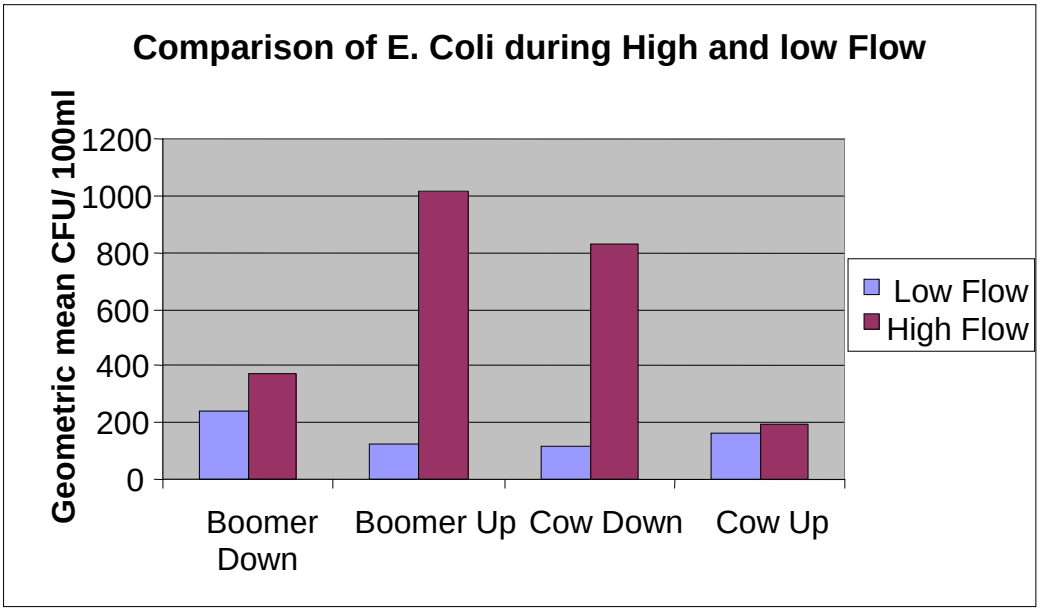

Figure 5-3: Comparison of E. coli during High Flow and Low Flow by location

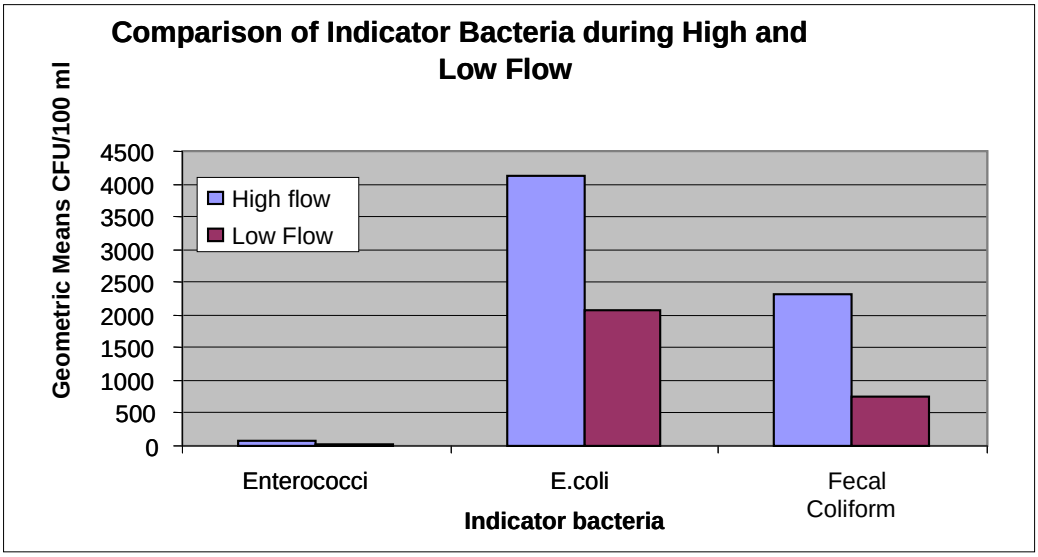

Figure 5-4: Comparison of Indicators during High and Low Flows (fecal coliform excludes E. coli), all locations combined

In urban areas, higher geometric means for all indicator organisms were observed during the high flow period as shown in Figure 5-5. In the rural areas, all indicator organisms had higher geometric means during high flow as well. In addition, *E. coli* and *Enterococci* exceeded water quality standards during both high and low flows. Total Fecal coliform exceeded U.S. EPA standards during high flow (Figure 5-6). In the urban areas, both *E. coli* and Total fecal coliform exceeded USEPA recommended standards during both low and high flows. *Enterococci* and fecal coliform violated water quality standards only during high flow.

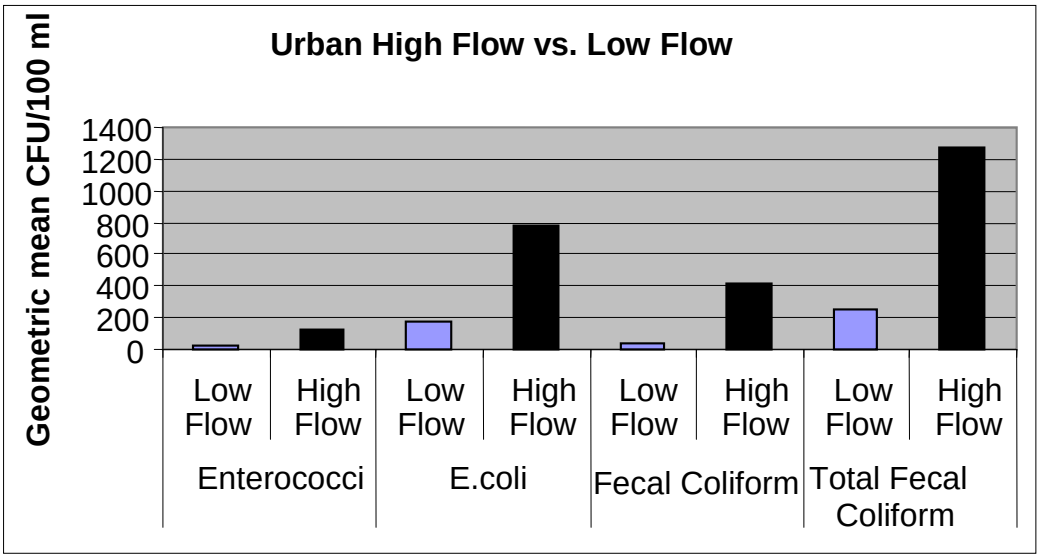

Figure 5-5: Urban High vs. Low Flow (fecal coliform excludes E. coli)

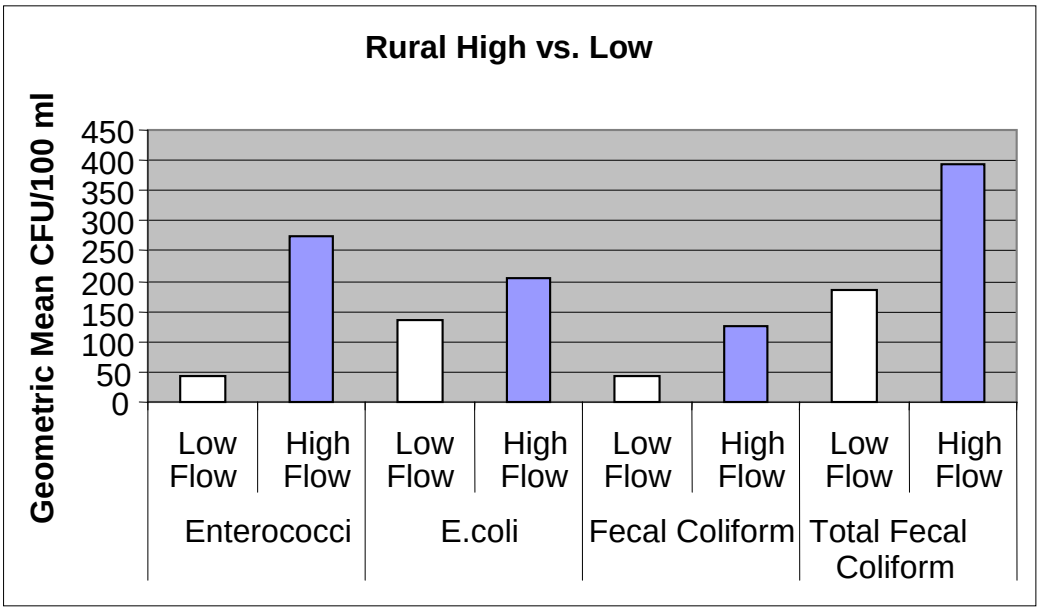

Figure 5-6: Rural High vs. Low Flow (fecal coliform excludes E. coli)

Land Uses

The sites sampled on Cow Creek are more rural than Boomer Creek. Both areas showed high levels of indicator organisms from time to time. However, there was not a consistent tendency for Cow Creek to be higher than Boomer Creek as hypothesized. The indicator organisms registered variations from date to date and among locations. Cow Creek would be likely to receive contamination from livestock or farming activities, particularly downstream. The highest concentrations of indicator bacteria were found downstream from the Oklahoma State University Swine facility, Equine Center and Dairy facilities. There were also swine located less than 50 yards above the downstream sampling location. In most cases, fecal indicator bacteria were highest downstream in the water column during high flow, which is much more affected by precipitation. The area is not highly developed, so wildlife and livestock may contribute to the fecal bacteria.

Boomer Creek is located in a more developed area. The majority of the creek is surrounded by residential and commercial buildings. The majority of samples taken on Boomer Creek during high flow had high numbers of all indicator organisms, especially upstream. This may be due to the effects of Boomer Lake, a recreational area that individuals use for walking pets. There is also an abundance of geese and other water fowl. Pets along with other urban wildlife are likely the contributors of bacterial pollution. If animals are the main contaminant source, it is important for cities to monitor animal waste (livestock and wildlife).

Total fecal coliform, *E. coli* and fecal coliform were generally higher in the urban areas rather than the rural areas. However, the test of land use on fecal coliform wasn't significant with a p-value of 0.26. *Enterococci* had p-value of 0.06 for the test of land

uses, with rural higher than urban which is considered marginally significant (Figure 5-7). Nearly all indicator organisms in the urban and rural areas exceeded their respective water quality standards on average.

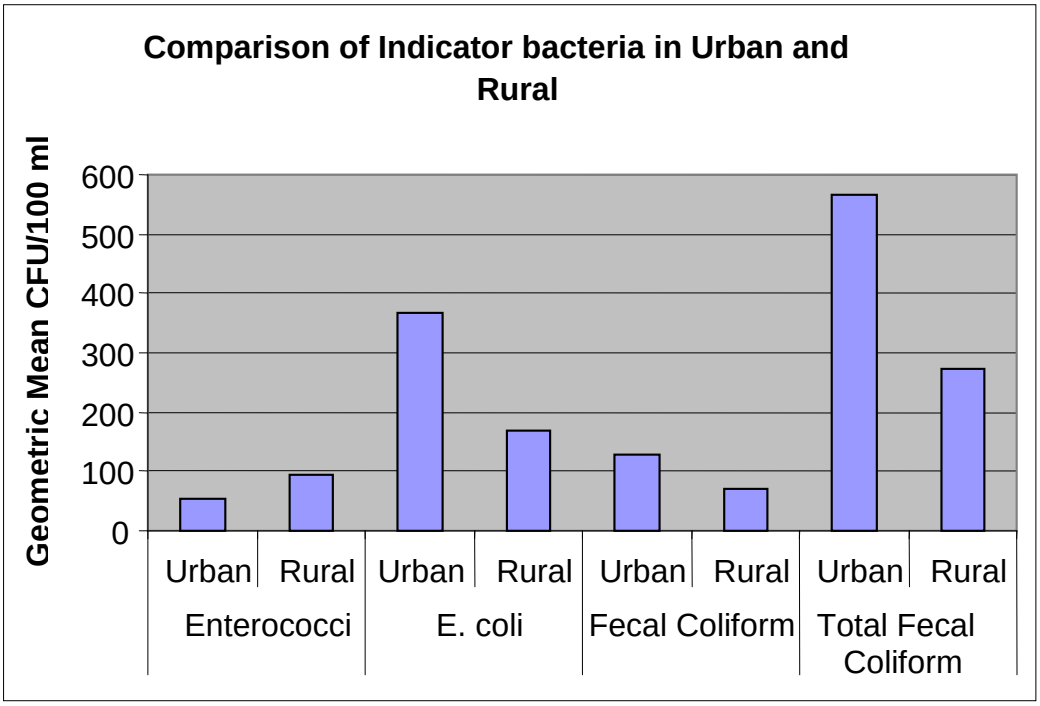

Figure 5-7: Comparison of Indicator organisms between Urban and Rural (fecal coliform excludes E. coli)

FC/EC Ratio

The FC/EC ratios for water column samples were calculated by dividing Total fecal coliform by *Enterococci* for evidence that samples were from more of a human or non-human origin. A ratio greater than 4 is considered to be from a human source (Sargent 1999). Non-human sources should exhibit ratios less than 0.7. On June 12, 2007, Boomer Creek had high FC/EC ratio in both upstream and downstream locations. This was presumed to be from human origins. On Cow Creek, the upstream had a high ratio, but downstream had an FC/EC ratio lower than 0.07, which is considered to be more from an animal source (Table 5-2).

On July 9, 2007, FC/EC ratios were low at both upstream and downstream location at Boomer Creek as shown in Table 5-2. Cow Creek also had low FC/EC ratio in both locations. Samples from Boomer Creek on July 22, 2007 had higher FC/EC upstream. Both upstream and downstream were presumed to be of animal origin (Table 5-2). Cow Creek had similar values at both the upstream and downstream sites. On August 6, 2007, Boomer Creek had high FC/EC concentration in both upstream and downstream. These were presumed likely to be of human origin. At Cow Creek, both locations had low FC/EC ratios (Table 5-2).

Table 5-2: FC/EC ratio for Water Column Samples

Stations	High Flow		Low Flow	
Dates	6/12/07	7/10/07	7/22/07	8/6/07
Boomer up	19.9 H	0.669 N	1.25	4.58 H
Boomer Down	5.08 H	0.788	0.154 N	9.06 H
Cow up	11.8 H	0.704	1.92	0.827
Cow Down	1.322	0.355 N	0.371 N	1.29

*H= presumed human source
*N= presumed non-human source

Conclusion

This study analyzed the distribution and occurrence of *E. coli*, *Enterococci* and fecal coliform as affected by land use, sample type and flow. At all four sites, all indicator organisms were detected at high density more often during high flow. During low flow, *E. coli* showed more of a consistent tendency of exceeding water quality standards. Water quality standards are geared more toward monitoring water column and low flow. *E. coli* and fecal coliform were found to be highly correlated, but there was not a significant correlation between either *E. coli* or fecal coliform and *Enterococci*. The correlation of E. coli and Total fecal coliform was significant. Therefore, measuring either concentration should allow for reliable prediction of the other.

The main objective of this study was to determine the concentration of indicator bacteria in the water column and sediments under different land uses and flow regimes. All sites sampled during wet weather in urban and rural areas displayed elevated concentration of indicator bacteria for both water column and sediment samples generally exceeding low-flow water quality standards. During the sampling period there was a significant amount of rain received as shown in Appendix C. Indicator bacteria were highest under high flow at all location with the exception of *Enterococci*. During dry weather conditions indicator bacteria were below the water quality standard for primary body contact in both urban and rural areas.

The aim of this study was to help find more effective indicator for detection of a risk of pathogens in water. There was a greater consistency of *Enterococci and E. coli* to violate EPA recommended standards. Due to short term and limited scope of the study, however we were unable to determine which indicator organism is the best for monitoring urban and rural land uses. There is a strong correlation between *E. coli* and fecal coliform. I recommend that E. coli be used as an indicator instead of fecal coliform, because *E. coli* is considered a better indicator of possible illness to swimmers. I also recommend *Enterococci* as an indicator because these organisms are known to be more human specific. Especially in marine water these organisms should be used because they are able to survive in salty water. No single organism is expected to monitor all pathogens in waters. I suggest a combination of *E. coli* and *Enterococci* to monitor pathogen in streams. Both organisms are can be enumerated by the IDEXX method and membrane filtration technique. Testing for these organisms may be time consuming and often difficult. Methodology should be chosen depending on budget and intended use.

Recommendation for Future Research

There is need for a simple single standard method for fast detection of disease causing pathogens in recreational waters. This method must be simple and consistent and less time consuming for obtaining results. Rapid detection of fecal bacteria will remain a crucial issue for public health of people participating in water-related recreational activities.

These research results showed that both urban and rural landscapes had elevated concentration of indicator organisms from time to time within the study sites. The sources of microbial contamination were not pinpointed to an exact source. Future studies are needed to determine the exact sources of these indicator organisms.

Additional research is needed to evaluate other land uses such as industrial, agricultural, and commercial as they may be detrimental to the quality of water. I recommend that more sampling dates be analyzed to get a greater range of indicator bacteria concentrations. This study was only done during the summer months. I also recommend that samples be taken during all seasons to determine the concentration of the indicator organisms during different season.

Much more research is needed to completely address the issues involving our contaminated recreational waters. Progress has been made in public education and monitoring programs addressing fecal bacteria in contamination waters, but more is needed to improve public health and the wellbeing of people in recreational waters.

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7. Appendix

Appendix A Indicator Organisms Sampling Data

High flow
Water Column

Station	Date	E. coli		Fecal coliform		Entercocci	
	6/12/07	R1	R2	R1	R2	R1	R2
Boomer up		2280	1400	900	1700	21.3	18.3
Boomer Down		200	500	400	100	66.3	49.6
Cow up		130	0	780	0	17.1	29.8
Cow Down		2700	2000	90	120	83.6	689.3

Station	Date	E. coli		Fecal coliform		Entercocci	
	7/10/07	R1	R2	R1	R2	R1	R2
Boomer up		920	2440	360	430	360.9	960.6
Boomer Down		430	450	340	250	549.3	248.9
Cow up		450	121	230	310	157.6	913.9
Cow Down		430	200	560	180	960.6	829.7

*Fecal coliform excludes E. coli

Low flow

Station	Date	E. coli		Fecal coliform		Entercocci	
	7/22/07	R1	R2	R1	R2	R1	R2
Boomer up		100	180	40	30	34.5	22.3
Boomer Down		370	280	0	40	35	48
Cow up		200	290	40	110	32.3	36.8
Cow Down		50	20	10	30	55.4	39.3

Station	Date	E. coli		Fecal coliform		Entercocci	
	8/6/07	R1	R2	R1	R2	R1	R2
Boomer up		70	190	70	140	23	20.3
Boomer Down		210	150	120	140	13.2	15.5
Cow up		120	110	20	30	22.8	38.4
Cow Down		630	260	140	80	73.8	90.8

*Fecal coliform excludes E. coli

Sediment
High flow

Station	Date	E. coli		Fecal coliform		Entercocci	
	6/12/07	R1	R2	R1	R2	R1	R2
Boomer up		16000	12400	48000	36000	16	10.9
Boomer Down		4000	32000	3000	0	11	5.2
Cow up		0	56000	0	0	14.6	7.4
Cow Down		54000	20000	3000	4000	51.2	74.3

Station	Date	E. coli		Fecal coliform		Entercocci	
	7/10/07	R1	R2	R1	R2	R1	R2
Boomer up		0	0	0	0	17.1	68.7
Boomer Down		20000	990000	40000	80000	133.4	629.4
Cow up		90000	28000	3000	24000	106.3	100.8
Cow Down		91000	56000	20000	32000	90.8	689.3

*Fecal coliform excludes E. coli

Low flow

Station	Date	E. coli		Fecal coliform		Entercocci	
	7/22/07	R1	R2	R1	R2	R1	R2
Boomer up		40000	27000	1000	13000	2	14.6
Boomer Down		33000	21000	5000	7000	67.7	22.8
Cow up		40000	84000	30000	48000	10.7	107.1
Cow Down		56000	40000	35000	10000	43.5	67

Station	Date	E. coli		Fecal coliform		Entercocci	
	8/6/07	R1	R2	R1	R2	R1	R2
Boomer up		9000	37000	3000	15000	68.3	29.8
Boomer Down		6000	49000	45000	26000	12.2	13.5
Cow up		4000	24000	7000	1000	629.4	120.1
Cow Down		67000	47000	2000	18000	113.7	40.8

*Fecal coliform excludes E. coli

Appendix B Conformation of Enterococci

Enterococci Confirmation for Cow Creek

	Number of Isolate	Number confirmed
6/12/07	102	91
2/9/07	103	115
7/22/07	118	97
8/6/07	105	112

Enterococci Confirmation for Boomer Creek

	Number of Isolate	Number confirmed
6/12/07	74	65
2/9/07	99	81
7/22/07	70	63
8/6/07	74	70

Appendix C Rainfall Data

June'07	Rain (in)	July' 07	Rain (in.)	August '07	Rain (in)
1	5.32	1	0.37	1	0
2	0	2	0.05	2	0
3	0	3	0.08	3	0
4	0	4	0.04	4	0
5	0	5	0.35	5	0
6	0	6	0	6	0
7	0	7	0	7	0
8	0	8	0	8	0
9	0.01	9	0.68	9	0
10	0.39	10	0	10	0
11	0.00*	11	0	11	0
12	0.01	12	1.38	12	0
13	1.34	13	2.86	13	0
14	0.73	14	0	14	0
15	0.1	15	0	15	0
16	0.02	16	0	16	0
17	0.66	17	0	17	0
18	0	18	0	18	0.28
19	0.75	19	0	19	0.51
20	0.58	20	0	20	0
21	0	21	0	21	0
22	0	22	0	22	0
23	0.8	23	0.69	23	0
24	0	24	0.04	24	0.49
25	0.03	25	0	25	0.03
26	2.34	26	0	26	0
27	0.67	27	0	27	0
28	2.25	28	0	28	0
29	0.73	29	0	29	0
30	0.01	30	0.47	30	0.00*
		31	0	31	0
Total	16.74	Total	7.01	Total	1.31

Appendix D Statistical Analysis

Analysis of Water Data.
Means and Standard Errors of E_coli and LN_E_coli by Type*Flow*Stream*Location.

Obs	Type	Flow	Stream	Location	MN_E_coli	SE_E_coli	MN_log	SE_log
1	Column	High	Boomer	Down	395.00	66.65	5.9215	0.21011
2	Column	High	Boomer	Up	1240.00	406.61	6.9217	0.39184
3	Column	High	Cow	Down	1332.50	606.40	6.7160	0.62065
4	Column	High	Cow	Up	233.67	108.20	5.2575	0.42637
5	Column	Low	Boomer	Down	252.50	47.32	5.4765	0.19361
6	Column	Low	Boomer	Up	135.00	29.58	4.8234	0.24051
7	Column	Low	Cow	Down	240.00	140.53	4.7285	0.78052
8	Column	Low	Cow	Up	180.00	41.83	5.1140	0.22741
9	Sediment	High	Boomer	Down	261500.00	2 42901.04	10.5941	1.15933
10	Sediment	High	Boomer	Up	14200.00	1800.00	9.5529	0.12745
11	Sediment	High	Cow	Down	55250.00	14499.28	10.7880	0.31793
12	Sediment	High	Cow	Up	58000.00	17925.77	10.8602	0.33902
13	Sediment	Low	Boomer	Down	27250.00	9113.86	9.9639	0.45562
14	Sediment	Low	Boomer	Up	28250.00	6992.56	10.1060	0.34431
15	Sediment	Low	Cow	Down	52500.00	5838.09	10.8500	0.11123
16	Sediment	Low	Cow	Up	38000.00	17009.80	10.0788	0.64811

Analysis of Water Data.
Means and Standard Errors of Fecal_Coliform and LN_Fecal for TYPE.

Obs	Type	MN_Fecal_ Coliform	SE_Fecal_ Coliform	MN_LN_ Fecal	SE_LN_ Fecal
1	Column	259.67	64.06	4.87639	0.2237
2	Sediment	74148.15	39284.76	9.65862	0.31779

Analysis of Water Data.
Means and Standard Errors of Fecal_Coliform and LN_Fecal for FLOW.

Obs	Flow	MN_Fecal_ Coliform	SE_Fecal_ Coliform	MN_LN_ Fecal	SE_LN_ Fecal
1	High	67028.85	41267.25	7.74566	0.52868
2	Low	8614.19	2494.25	6.63508	0.50833

Analysis of Water Data.
Means and Standard Errors of Fecal_Coliform and LN_Fecal for STREAM.

Obs	Stream	MN_Fecal_ Coliform	SE_Fecal_ Coliform	MN_LN_ Fecal	SE_LN_ Fecal
1	Boomer	61037.86	38439.66	7.38443	0.54284
2	Cow	10370.00	3005.45	6.90726	0.51266

Analysis of Water Data.
Means and Standard Errors of Fecal_Coliform and LN_Fecal for LOCATION.

Obs	Location	MN_Fecal_ Coliform	SE_Fecal_ Coliform	MN_LN_ Fecal	SE_LN_ Fecal
1	Down	43953.33	32820.76	7.24110	0.52471
2	Up	25599.63	17663.55	7.03117	0.53280

Analysis of Water Data.
Means and Standard Errors of Enterococci and LN_Enter for TYPE.

Obs	Type	MN_Fecal_ MN_Enterococci	SE_Fecal_ SE_Enterococci	MN_LN_ Enter	SE_LN_ Enter
1	Column	204.944	55.5766	4.28743	0.24645
2	Sediment	105.925	32.1917	3.71876	0.24533

Analysis of Water Data.
Means and Standard Errors of Enterococci and LN_Enter for FLOW.

Obs	Flow	MN_Fecal_ MN_Enterococci	SE_Fecal SE_Enterococci	MN_LN_ Enter	SE_LN_ Enter
1	High	249.475	57.8509	4.44288	0.28751
2	Low	61.394	19.1289	3.56331	0.17607

Analysis of Water Data.
Means and Standard Errors of Enterococci and LN_Enter for STREAM.

Obs	Stream	MN_Fecal_ MN_Enterococci	SE_Fecal_ SE_Enterococci	MN_LN_ Enter	SE_LN_ Enter
1	Boomer	112.800	38.2903	3.60120	0.24896
2	Cow	198.069	51.9594	4.40499	0.23182

Analysis of Water Data.
Means and Standard Errors of Enterococci and LN_Enter for LOCATION.

Obs	Location	MN_Fecal_ MN_Enterococci	SE_Fecal_ SE_Enterococci	MN_LN_ Enter	SE_LN Enter
1	Down	184.816	48.4129	4.28926	0.24366
2	Up	126.053	43.4015	3.71694	0.2479

Analysis of Water Data.
Response variable is LN_E_coli.

Tests of Effect Slices

Effect	Type	Flow	Stream	Location	Num DF	Den DF	F Value	Pr >
Type*Flow*Stre*Locat	Column	High	Boomer		1	14.9	1.22	0.2868
Type*Flow*Stre*Locat	Column	High	Cow		1	17	2.54	0.1293
Type*Flow*Stre*Locat	Column	Low	Boomer		1	14.9	0.52	0.4818
Type*Flow*Stre*Locat	Column	Low	Cow		1	14.9	0.18	0.6763
Type*Flow*Stre*Locat	Sediment	High	Boomer		1	14.9	0.88	0.3627
Type*Flow*Stre*Locat	Sediment	High	Cow		1	17	0.01	0.9327
Type*Flow*Stre*Locat	Sediment	Low	Boomer		1	14.9	0.02	0.8774
Type*Flow*Stre*Locat	Sediment	Low	Cow		1	14.9	0.73	0.4077
Type*Flow*Stre*Locat	Column	High		Down	1	14.9	0.77	0.3941
Type*Flow*Stre*Locat	Column	High		Up	1	17	3.28	0.0877
Type*Flow*Stre*Locat	Column	Low		Down	1	14.9	0.68	0.4217
Type*Flow*Stre*Locat	Column	Low		Up	1	14.9	0.10	0.7526
Type*Flow*Stre*Locat	Sediment	High		Down	1	14.9	0.05	0.8333
Type*Flow*Stre*Locat	Sediment	High		Up	1	16.3	1.33	0.2652
Type*Flow*Stre*Locat	Sediment	Low		Down	1	14.9	0.96	0.3433
Type*Flow*Stre*Locat	Sediment	Low		Up	1	14.9	0.00	0.9764
Type*Flow*Stre*Locat	Column		Boomer	Down	1	14.9	0.24	0.6302
Type*Flow*Stre*Locat	Column		Boomer	Up	1	14.9	5.37	0.0351
Type*Flow*Stre*Locat	Column		Cow	Down	1	14.9	4.82	0.0444
Type*Flow*Stre*Locat	Column		Cow	Up	1	17	0.01	0.9193
Type*Flow*Stre*Locat	Sediment		Boomer	Down	1	14.9	0.48	0.4971
Type*Flow*Stre*Locat	Sediment		Boomer	Up	1	14.9	0.25	0.6252
Type*Flow*Stre*Locat	Sediment		Cow	Down	1	14.9	0.00	0.9463
Type*Flow*Stre*Locat	Sediment		Cow	Up	1	17	0.70	0.4142
Type*Flow*Stre*Locat		High	Boomer	Down	1	14.9	26.64	0.0001
Type*Flow*Stre*Locat		High	Boomer	Up	1	14.9	5.63	0.0315
Type*Flow*Stre*Locat		High	Cow	Down	1	14.9	20.23	0.0004
Type*Flow*Stre*Locat		High	Cow	Up	1	19.1	33.26	<.0001
Type*Flow*Stre*Locat		Low	Boomer	Down	1	14.9	24.57	0.0002
Type*Flow*Stre*Locat		Low	Boomer	Up	1	14.9	34.05	<.0001
Type*Flow*Stre*Locat		Low	Cow	Down	1	14.9	45.72	<.0001
Type*Flow*Stre*Locat		Low	Cow	Up	1	14.9	30.08	<.0001

Fecal Coliform

The four-way interaction term, Type*Flow*Stream*Location, is not significant $(\alpha = 0.05, F_{1,13.2} = 0.08, p.value = 0.77.$

The three-way interaction terms are also not significant:

Type*Flow*Stream $(\alpha = 0.05, F_{1,14} = 0.76, p.value = 0.39$
Type*Flow*Location $(\alpha = 0.05, F_{1,14} = 0.05, p.value = 0.81$
Type*Stream*Location $(\alpha = 0.05, F_{1,13.9} = 0.03, p.value = 0.86$
Flow*Stream*Location $(\alpha = 0.05, F_{1,14} = 0.23, p.value = 0.63.$

The two-way interaction terms are not significant:

Type*Flow $(\alpha = 0.05, F_{1,18} = 0.40, p.value = 0.53$
Type*Stream $(\alpha = 0.05, F_{1,18} = 0.00, p.value = 0.96$
Type*Location $(\alpha = 0.05, F_{1,17.9} = 0.06, p.value = 0.81$
Flow*Stream $(\alpha = 0.05, F_{1,18.2} = 0.57, p.value = 0.46$
Flow*Location $(\alpha = 0.05, F_{1,18} = 2.36, p.value = 0.14$
Stream*Location $(\alpha = 0.05, F_{1,18} = 0.09, p.value = 0.76.$

The main effects Type and Flow are highly significant
$(\alpha = 0.05, F_{1,23.5} = 149.25, p.value < 0.0$ and
$(\alpha = 0.05, F_{1,23.7} = 15.12, p.value = 0.00,$ respectively.

The other main effects are not significant:

Stream $(\alpha = 0.05, F_{1,23.6} = 1.37, p.value = 0.26$
Location $(\alpha = 0.05, F_{1,23.6} = 0.41, p.value = 0.52.$

Enterococci

The four-way interaction term, Type*Flow*Stream*Location, is not significant $(\alpha = 0.05, F_{1,16} = 0.04, p.value = 0.84.$

The three-way interaction terms are also not significant:

Type*Flow*Stream $(\alpha = 0.05, F_{1,17} = 0.11, p.value = 0.74$
Type*Flow*Location $(\alpha = 0.05, F_{1,17} = 0.07, p.value = 0.79$
Type*Stream*Location $(\alpha = 0.05, F_{1,17} = 0.38, p.value = 0.64$
Flow*Stream*Location $(\alpha = 0.05, F_{1,17} = 0.30, p.value = 0.59.$

The two-way interaction terms are not significant:

Type*Flow $(\alpha = 0.05, F_{1,21} = 2.77, p.value = 0.11$
Type*Stream $(\alpha = 0.05, F_{1,21} = 0.35, p.value = 056$
Type*Location $(\alpha = 0.05, F_{1,21} = 0.03, p.value = 0.87$
Flow*Stream $(\alpha = 0.05, F_{1,21} = 0.22, p.value = 0.64$
Flow*Location $(\alpha = 0.05, F_{1,21} = 1.05, p.value = 0.31$
Stream*Location $(\alpha = 0.05, F_{1,21} = 0.25, p.value = 0.62.$

The main effect, Flow, is significant $(\alpha = 0.05, F_{1,27} = 4.49, p.value = 0.04.$

The other main effects are not significant:

Type $(\alpha = 0.05, F_{1,27} = 1.88, p.value = 0.18$
Stream $(\alpha = 0.05, F_{1,27} = 3.75, p.value = 0.06$
Location $(\alpha = 0.05, F_{1,27} = 1.90, p.value = 0.17.$

Vita

Adrian Tyrone Sherman

Candidate for the Degree of

Master of Science

Thesis: THE OCCURRENCE AND DISTRIBUTION OF FECAL INDICATOR BACTERIA WITH RESPECT TO URBAN AND RURAL LAND USES

Biographical:

Education: Received a Bachelor of Science Degree in Natural Resource Management at Langston University in Langston, Oklahoma in 2005. Completed the Requirements for the Master of Science Degree with a major in Environmental Science at Oklahoma State University in May, 2009.

Experience: Employed as a reality aid for the Bureau of Land Management; employed by Oklahoma State University in Plant Pathology as Lab Technician; employed by Oklahoma State University as a graduate Assistant in Biosystems and Agriculture Dept.2005 to December 2008 Employed as Environmental Specialist at the Oklahoma Department of Environmental Quality.

Professional Memberships: Golden Key International Honuor Society, Alpha Chi, Beta Kappa Chi.

Name: Adrian Sherman Date of Degree: May, 2009

Institution: Oklahoma State University Location: Stillwater, Oklahoma

Title of Study: OCCURRENCE AND DISTRIBUTION OF FECAL INDICATOR BACTERIA WITH RESPECT TO URBAN AND RURAL LAND USES

Pages in Study: 70 Candidate for the Degree of Master of Science

Major Field: Environmental Science

Scope and Method of Study: There are many streams in Oklahoma that are vulnerable to fecal contamination from a variety of sources. The primary objective of this study was to determine the relative distribution of alternative indicator species such as *Enterococci*, *Escherichia coli* and Fecal Coliform as affected by land use (urban and rural), flow regime (high and low flow) and sample type (sediment and water column). The tests were conducted on Boomer and Cow Creeks, which are tributaries of Stillwater Creek, located in Stillwater, Oklahoma. The two locations on Boomer Creek represented urban land use, and the two on Cow Creek represented rural land use. There were also samples taken during baseflow and stormflow to compare microbial indicators during different flow regimes. Samples were processed using Enterolert method (*Enterococc*i) and membrane filtration method (*E. Coli* and Fecal Coliform). Correlations were performed to understand the relationship between indicator organisms. The study provided insight on potential sources of fecal contamination.

Findings and Conclusions: At all sampling sites, indicator organisms were detected at high density, especially during high flow and in sediments. There was not a consistent trend of greater densities of indicator organisms found in rural or urban areas. There was a consistent trend of *E. coli* and *Enterococci* violating recommended standards. In addition, strong correlations were found between *E. coli,* total fecal coliform and fecal coliform. The correlation between *E. coli* and total fecal coliform were highly significant suggesting that one organism may predict the other. *Enterococci* correlations were not significant with any of the coliforms.

ADVISER'S APPROVAL: Dr. Michael D. Smolen